BUTTERFLY EXPLOITATION OF AN ANT-PLANT MUTUALISM: ADDING INSULT TO HERBIVORY

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Abstract. —The myrmecophilous butterfly caterpillar of *Thisbe irenea* is shown to gain growth benefits from not only feeding on leaf tissue, but by also drinking the extrafloral nectar of its hostplant. Since both the plant and caterpillar use ants as defenses, it is suggested that a conflict is generated between plant and herbivore for the attentions of ants, and that such conflicts may be widespread in ant–plant, and ant–herbivore systems. It is further suggested that this study points to the possibility that in such systems, 2-species mutualisms may be susceptible to invasion and exploitation by a third species.

It is well documented that both plants and insect herbivores may form mutualisms with ants. Ants provide plants with protection against herbivores (see reviews in Buckley, 1983a; Beattie, 1985; Koptur, 1984), and ants provide insects with benefits that include protection against predators and parasitoids, faster growth rates and higher reproductive success (Banks and Nixon, 1958; Bartlett, 1961; Bristow, 1984; Cottrell, 1984; DeVries, 1987; Pierce et al., 1987). In these mutualisms insects provide ants with secretions directly through specialized organs (Mittler, 1958; Way, 1963; Cottrell, 1984; Fiedler and Maschwitz, 1988; DeVries, 1988; Wilson, 1971), whereas plants may either provide secretions directly through extrafloral nectaries (Beattie, 1985), or may attract ants indirectly via honey-dew secreting Homoptera (Messina, 1981).

Among butterflies the habit of associating with ants, or myrmecophily, is best known from the Lycaenidae whose larvae have specialized ant-organs for associating with ants (Cottrell, 1984). Larval ant-organs are so widespread within the Lycaenidae that myrmecophily is thought to have played an important role in lycaenid evolution (Hinton, 1951; Vane-Wright, 1978; Pierce, 1984). Some species in the Riodinidae also have larvae that associate with ants and possess ant-organs that are analogous, but not homologous, to those found on lycaenids (Cottrell, 1984; DeVries, 1988). Because they are considered to share a close relationship to the lycaenids (Ehrlich, 1958; Kristensen, 1976; Harvey, 1987; but see Robbins, 1988), assumptions about the evolution of myrmecophily in riodinids are based primarily on studies of lycaenids (Pierce, 1987). However, the biology of ant association in most riodinid species is unknown (see Ross, 1966; Callaghan, 1986; DeVries, 1987).

This paper presents observations and experiments done that probe hostplant use and ant association in the myrmecophilous riodinid butterfly *Thisbe irenea* (Stoll), and extends these observations to other ant–insect systems. The purpose of this paper is to show that some ant-associated caterpillars not only feed on plant tissues but also feed from extrafloral nectaries on the hostplant, thus exploiting the basis of the mutualism between plants and ants. We suggest that when plants and caterpillars

both have mutualistic associations with the same species of ants, a conflict is generated between plant and herbivore for the attentions of ant mutualists, and that such conflicts may be widespread in many ant–plant, and ant–herbivore systems. Although traditionally ant–plant mutualisms and ant–insect mutualisms have been considered separately, this study points to the possibility that 2-species mutualisms may be susceptible to invasion and exploitation by a third species.

MATERIALS AND METHODS

From September 1985 to September 1986, and intermittently from August 1987– October 1988, one of us (PJD) studied the wide ranging riodinid butterfly *T. irenea* on Barro Colorado Island, Panama, and on surrounding mainland habitats. Here *T. irenea* caterpillars feed only on saplings and seedlings of the euphorbiaceous pioneer tree *Croton billbergianus* (Robbins and Aiello, 1982; DeVries, 1987). The vegetative surfaces of the hostplant are patrolled by ants attracted to EFN's located at the base of each leaf, and these ants tend *T. irenea* caterpillars. All five larval stages feed on developing leaves, but only fourth and fifth instar caterpillars can eat all types of leaves; earlier instars must feed on new developing leaves. Infestations of *T. irenea* caterpillars commonly remove from 18–38% of the total leaf area of small *C. billbergianus* plants, occasionally killing them (DeVries, unpublished).

Upon reaching third instar, specialized ant-organs become functional that allow caterpillars to attract and maintain the presence of ants (DeVries, 1988). The major ant species tending both *T. irenea* and the EFN's of *C. billbergianus* at the study site was *Ectatomma ruidum* (Formicidae: Ponerinae), and these ants protect larvae from predators in exchange for secretions provided by ant-organs (DeVries, 1987).

From weekly and bi-monthly censuses of marked *C. billbergianus* plants, and observations on potted plants in an ambient temperature laboratory, it was established that *T. irenea* caterpillars of all instars rest with their heads on or immediately adjacent to EFN's of the hostplant (Fig. 1). First through third instar caterpillars spend most of their time, day and night, on or near EFN's, whereas fourth and fifth instars hide on the stem during the day and crawl up on the leaves to feed at night. The habit was observed so regularly as to suggest that caterpillars were drinking extrafloral nectar.

The following experiments were conducted to determine whether the presence of EFN's and ants affected larval growth. Twelve potted hostplants (paired by size, leaf number and leaf maturity) were ringed near their bases with Tanglefoot (Trade mark) to eliminate access to the foliage by crawling insects. One half of the plants had the EFN's excised, the other half did not. Pairs of plants were placed on either side of six captive colonies of *E. ruidum* ants maintained in plastic tubs. Ants from the colonies were allowed access to the plants by placing wooden bridges above the Tanglefoot into the plastic tub. Each plant received one larva of *T. irenea*; all larvae were the same instar and weight. Every 48 hours during the following 12 days caterpillars were weighed to the nearest milligram. A simultaneous experiment was set up in the same manner, except that larvae were grown without allowing ants to tend them. All experiments were done in an ambient temperature laboratory.

To examine if larvae drank nectar, and if nectar quality had an effect on growth, larvae were grown using experimental nectars without ants. Experimental nectaries were made from capillary tubes sealed into the sides of Petri dishes (DeVries, 1987).
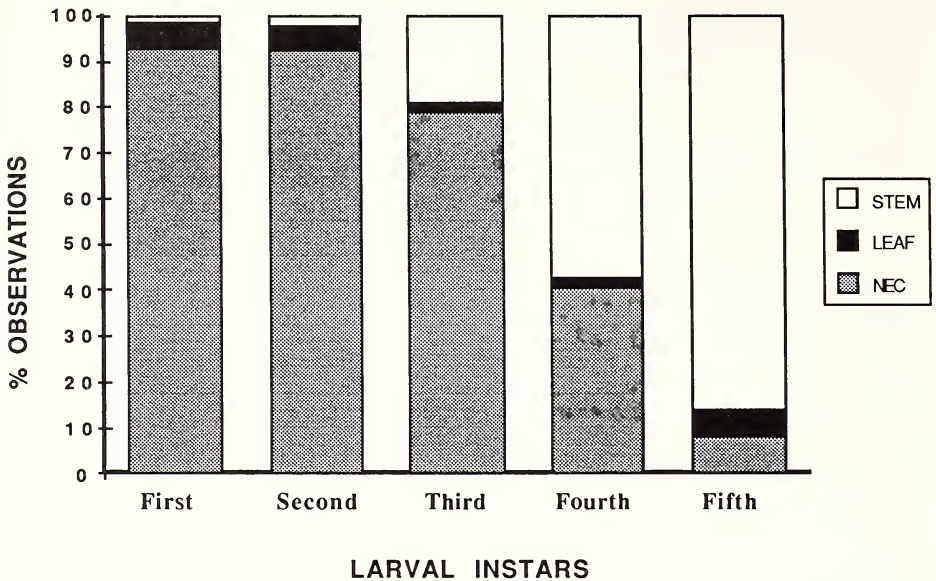
LARVAL INSTARS

Fig. 1. Summary of 1,378 diurnal observations of *Thisbe irenea* caterpillars taken from diurnal censuses. NEC = caterpillars found with head on or immediately next to an EFN; LEAF = caterpillars found on leaf tissue; STEM = caterpillars found resting on woody plant parts away from leaves. Late fourth and all fifth instar caterpillars rest on the stem during the day, but are active at night when they are frequently found with their heads on or near EFN's.

Nectars were made by adding 5 drops of red liquid food coloring to 400 ml of distilled water. They were then mixed well, and fractioned into two containers. To one container, 20% sucrose (by volume) was added to mimic extrafloral nectar. This was designated the experimental nectar. The other container, the control nectar, did not have added sucrose. Twenty four larvae, paired by weight and instar, were given standardized leaf sections and placed separately in Petri dishes fitted with experimental nectaries. Twelve larvae were placed in dishes fitted for experimental nectar, twelve were placed in dishes fitted for control nectar. All were kept in a constant humidity chamber. Every 24 hours the nectars were withdrawn and replenished, and every 48 hours larvae were weighed. Consumption of fluid was measured as millimeters traveled down the capillary tubes every 24 hours.

The effects of EFN's, ants, and nectar quality on larval growth through time were analyzed using a 3 way or 2 way Repeated Measures Design ANOVA (Winer, 1971). The factors in the analyses were: time, EFN's, and presence of ants for the 3 way analysis, and time and type of nectar for the 2 way analysis. Volumes of experimental and control nectar consumed were compared using a two-tailed, paired *t*-test (Sokal and Rohlf, 1981).

Ants often tended *T. irenea* larvae with greater frequency and fidelity than they did the EFN's of the hostplant (DeVries, 1988), suggesting that larvae are more attractive to ants than EFN's. To compare contents of caterpillar secretion and plant

extrafloral nectar, sample secretions were taken with micropipettes and spotted on chromatography paper. Amino acid and sugar concentrations were then analyzed using flourometric methods (Baker and Baker, 1976).

RESULTS

Two results suggested that *T. irenea* caterpillars drank extrafloral nectar and gained growth benefits from it (Fig. 2). First, caterpillars raised on plants with natural EFN's gained weight significantly faster (F[5,100] = 6.107, P < .0005) than those raised on plants with EFN's removed (Fig. 2a). The presence of ants also contributed to weight gain (F[5,100] = 2.821, P < .05), an effect also documented for other ant associated insects (Way, 1963). However, the presence of both ants and EFN's produced no significant increase in growth rate above the level achieved in the presence of EFN's alone (F[5,100] = 1.114, P = .358). Second, caterpillars raised with experimental nectars rested with their heads on or near the end of the artificial nectary, and recovery of food coloring in frass and epidermis demonstrated that caterpillars imbibed the nectar. Caterpillars raised with artificial nectar containing sugar imbibed significantly more fluid (paired-t [10] = 6.897, P < .005), and increased weight significantly faster (F[6,132] = 8.241, P < .0005) than those raised with the control solution containing no sugar (Fig. 2b).

The mean concentrations in 15 of 17 amino acids examined, as well as the total amino acid concentration (DeVries, 1988) were significantly higher in *T. irenea* caterpillar secretions than in extrafloral nectar of the hostplant (Fig. 3). Glutamic acid and methionine were found as non-measurable traces in caterpillar secretion, and in extrafloral nectar occurred at concentrations of .011 and .004 micrograms/microlitre respectively.

T. irenea secretions contained almost no sugars, in contrast to the high concentrations found in extrafloral nectar of *C. billbergianus* (DeVries, 1988). Since these caterpillars feed at EFN's but do not secrete sugars to ants, this suggests that caterpillars metabolize the sugars taken in as extrafloral nectar, but that sugar plays a minor role in caterpillar secretion as an ant attractant.

DISCUSSION

We have shown that, in addition to feeding on leaf tissue, myrmecophilous larvae of *T. irenea* gain growth benefits from drinking the extrafloral nectar of their hostplant (Fig. 2). Drinking extrafloral nectar explains why these larvae typically rest with their heads on or near EFN's (Fig. 1). Since the ant-organs, which become functional in the third instar, attract and maintain protective ants (DeVries, 1988), the contribution of extrafloral nectar to accelerated growth should greatly benefit young caterpillars by permitting them to reach the third instar quickly.

Our results show that the larval section of *T. irenea* contains significantly higher amino acid concentrations than the extrafloral nectar of *C. billbergianus* (Fig. 3), but that larval secretion contains almost no sugars. Thus, the high amino acid content of larval secretion is likely to be a factor influencing the preference of *E. ruidum* ants for tending *T. irenea* caterpillars over the EFN's of *C. billbergianus* (DeVries, 1988). In contrast to *T. irenea,* some lycaenid caterpillar secretions may have amino acid and sugar concentrations similar to extrafloral nectar (Maschwitz et al, 1975; Pierce,

Effects of nectar and ants on Thisbe irenea larvae
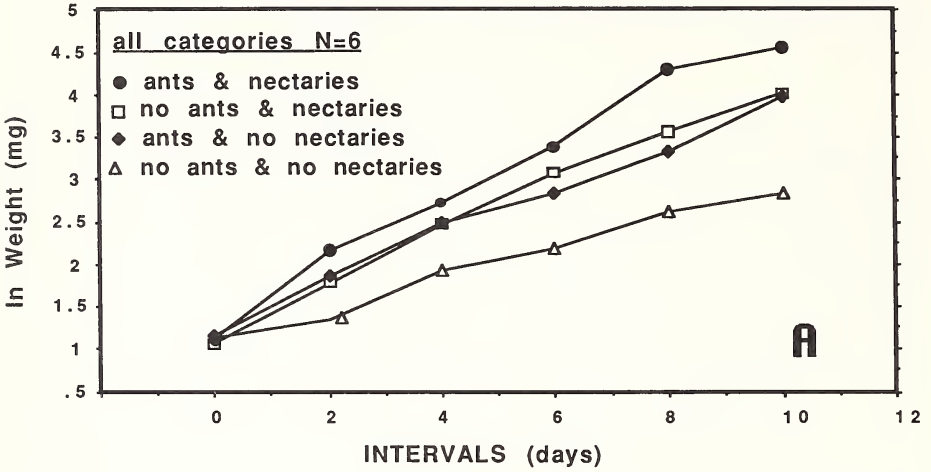
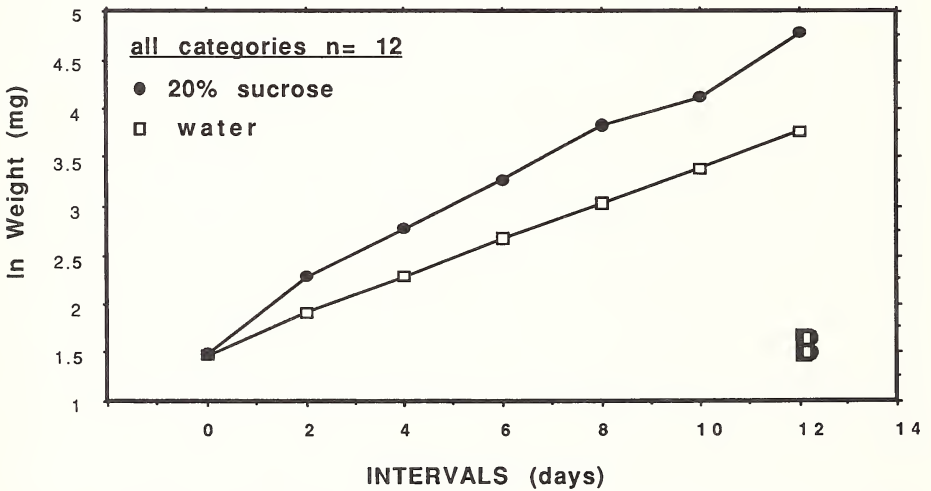**Effects of experimental nectar on Thisbe irenea larvae**

Fig. 2.   Summary of the growth responses of *Thisbe irenea* caterpillars to EFN's, ants, and experimental nectars. A. Caterpillar grown on potted plants with captive ant colonies. B. Caterpillars grown without ants on standardized leaf tissue and experimental nectars.

1983). Different ant taxa may show demonstrable preferences for different amino acid and sugar concentrations (Lanza and Krauss, 1984; Lanza, 1988), and recently a broad taxonomic pattern of caterpillar–ant associations was shown to be explained, in part, by the feeding ecology of ants (DeVries, 1987). We suggest that further insights
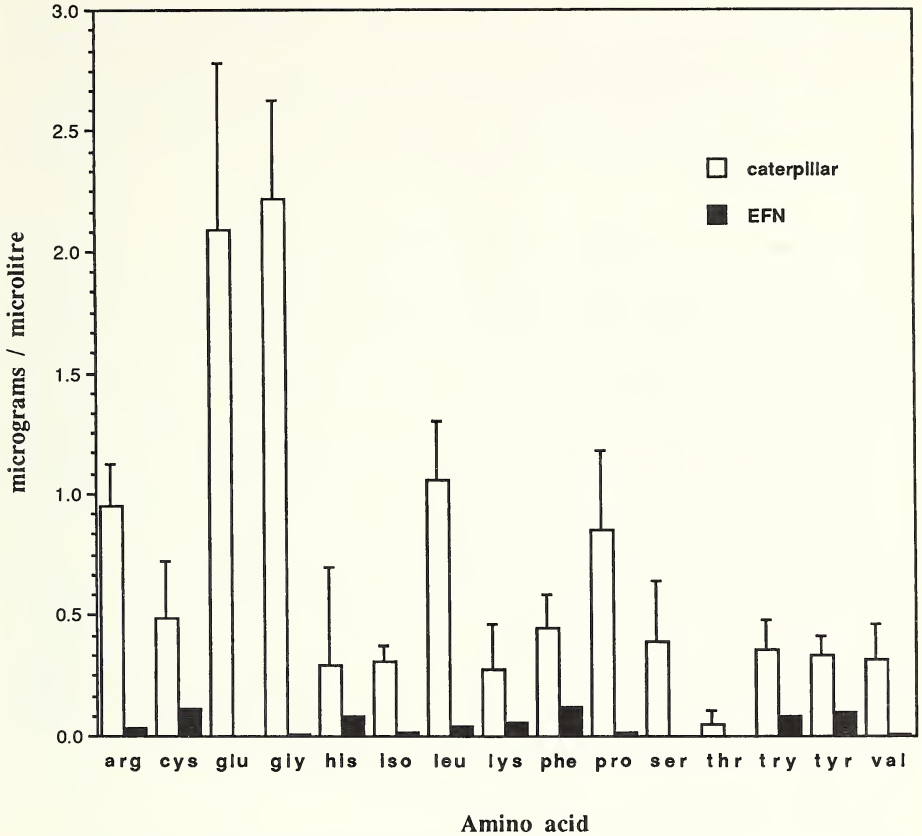
Figure 3.   Concentrations in micrograms per microlitre of 15 amino acids in caterpillar secretion (N = 5) and extrafloral nectar (N = 4). Concentrations of amino acids varied among individual caterpillars, but not among individual plants (DeVries, 1988).

into the taxonomic patterns of caterpillar–ant associations may be gained by analyzing secretions from many species of caterpillars. For example, butterfly caterpillars like *T. irenea* that associate with ants in a subfamily comprised of predaceous species may produce secretions with a nutrient content that approximates arthropod prey items — high amino acid concentrations and low sugar content. In contrast, caterpillar species associating with ants that typically harvest secretions (e.g., *Azteca* spp, *Iridomyrmex* spp. [Dolichoderinae], *Camponotus* spp. *Oecophylla* spp. [Formicinae]) may produce secretions with amino acid and sugar concentrations similar to extrafloral nectar.

A theoretical understanding of both ant–plant and ant–insect mutualism results from considering how two-species mutualisms evolve and are maintained (May, 1976; Goh, 1979; Addicott, 1981; Pierce and Young, 1986). However, the butterfly caterpillars described here use the mutualism between plants and ants, and the basis of this mutualism for their own benefit: both ants and extrafloral nectar benefit the

growth and survival of herbivorous caterpillars. While we cannot reject the possibility that this system is a 3-way mutualism (i.e., that plant, ant, and caterpillar all benefit from association), it is unlikely that substantial loss of leaf area to caterpillars benefits the plant (DeVries, unpublished). Hence, this study suggests that a third species has invaded a 2-species mutualism. Similar invasions are likely to occur between ant-plant mutualisms and herbivores because many ant-attracting insect herbivores feed on plants with EFN's.

Our current understanding of many ant-plant mutualisms suggests that ants protect plants from herbivores (Beattie, 1985; Atsatt and O'Dowd, 1976). Mutualism can occur between ant-attracting Homoptera and plants without EFN's because Homoptera may act as surrogate EFN's (Messina, 1981). However, mutualism is unlikely to occur between plants with EFN's and insects that attract ants (Buckley, 1983b); especially those insects with chewing mouthparts. When lycaenid caterpillars not only attract ants as defenses for themselves, but also specialize on new shoots or young leaves of plants with EFN's (Pierce, 1985), we suggest that a potential conflict is generated between plant and herbivore for the attention of ants; the plant stands to lose meristems and future photosynthetic potential to an herbivore invading a two-species mutualism. In ant associated riodinid butterflies the conflict between plant and herbivore may be stronger because their larvae commonly feed on extra-floral nectar in addition to leaf tissue (DeVries, 1987, and unpublished). These caterpillars not only feed on young meristematic tissues and benefit by using the plant's ant-guards for protection, but they also exploit the currency of the plant–ant mutualism (extrafloral nectar), thereby adding insult to herbivory. Thus, although it is typical to consider the evolutionary stability of two-species mutualism only in the context of both species, our findings suggest that two-species mutualisms may be vulnerable to invasion and exploitation by a third species.

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